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Antibacterial Application of Undoped and Doped CdO Nanocrystalline Thin Films

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Abstract

Nano crystalline thin films of undoped and Al doped Cadmium oxide were synthesized using chemical bath deposition method and were annealed at 500˚C. The films were characterized to study their structural, optical and compositional properties. Antibacterial activity of CdO films were assayed by using the agar well diffusion technique. The antibacterial activity of undoped and Al doped CdO solutions was analyzed to the Gram-positive bacteria Bacillus cereus and the gram-negative bacteria Vibrio Cholera. The present study reveals that the diameter of zone of inhibition is found to be more for gram-negative bacteria than gram-positive for undoped CdO and Al doped CdO.

Keywords : *Ammonium hydroxide; Cadmium chloride.*

1. INTRODUCTION

A number of species of bacteria are pathogenic and cause infectious diseases, including cholera, syphilis, leprosy, and plague. The most common deadly bacterial diseases are respiratory infections, with tuberculosis killing about 2 million people per year. In developed countries, antibiotics are used to treat bacterial contamination and are also used in farming. In industry, bacteria are important in sewage treatment and the breakdown of oil spills, the production of cheese and yogurt through fermentation, and the recovery of gold, palladium, copper and other metals in the mining sector.

Gram stain also called **Gram's Method**, is a method of staining used to discriminate bacterial type into two large groups namely gram-positive and gram-negative. A Zone of Inhibition Test also called a Kirby-Bauer Test is a qualitative method used clinically to measure antibiotic variance and technologically to ensure the potential of solids and textiles to inhibit microbial growth. A bacterial strain of significance is grown in pure culture which is spread over the face of a sterile agar plate. The antimicrobial agent is applied to the center of the agar plate. If the antimicrobial agent percolates from the object into the agar and then puts forth a growth-inhibiting effect, then a clear zone called the zone of inhibition appears around the test product. The size of the inhibition zone is usually associated to the level of antimicrobial activity present in the sample or product - a larger zone of inhibition typically means that the antimicrobial is more potent and powerful.

Bacterial strains of Gram-negative Vibrio and Gram-positive bacteria Bacillus cereus were used in this study. Vibrio is a type of Gram-negative bacteria, having a curved-rod shape, several kinds of which can root food borne infection, usually associated with eating undercooked seafood. Bacillus cereus is a Gram-positive, rod-shaped, aerobic, facultative anaerobic, beta hemolytic bacterium commonly found in soil and food.

2. EXPERIMENT

In the present work, Cadmium Oxide (CdO) thin films were prepared on glass substrates by sol-gel chemical bath deposition technique. In a conical flask, 0.1M of Cadmium chloride ($CdCl_2$ .2½H_2O) was dissolved in 250ml of deionized water. The solution was continuously stirred by a magnetic stirrer for 1hour to get a clear homogeneous solution. Ammonium hydroxide ($NH_3.H_2O$) solution was added with this solution drop wise till the pH is reached 12. The solution is taken in small beakers and the glass substrates were immersed into beakers for 24 hours. The glass slides were dried in hot air oven and were annealed to 500 °C. The undoped CdO thin film was prepared. The same procedure was continued to dope

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aluminum to CdO. Anhydrous aluminum chloride ($AlCl_3$) was added to the pure CdO precursor in the beaker (Ramiz Ahmed Al-Ansari, 2016) for different concentrations. The obtained results for the lesser concentrations of 1% and 2% of Al doping exhibit similar results for XRD analysis. Thus the concentration 3% and 5wt % were optimized. This solution was stirred using a magnetic stirrer for 2 hours and the glass substrates were dipped into the beaker for 24 hours.

Molecular grade chemicals were used for the experiment. Throughout the experiment, deionized water was used. Gram-positive bacteria, Bacillus cereus and Gram-negative bacteria, Vibrio cholerae were used for the present experiment. Nutrient Broth (Sigma) was used in growing and maintaining the bacterial cultures. CdO nano thin films, 3wt % Al doped CdO films and 5 wt% Al doped CdO films were used throughout the procedure. The films were suspended in ethanol individually and used.

3. X-RAY DIFFRACTION OF PURE CDO THIN FILMS

The structural properties of CdO nano particles were investigated using an X-ray diffraction analysis. The X-ray diffraction patterns were used to identify the structure and to calculate the particle size of synthesized Cadmium Oxide nano particles. The lattice parameter was calculated from the following Eq. (1):

$$a = d\sqrt{h^2+k^2+l^2}\ A^\circ \qquad (1)$$

where d is the spacing between adjacent (hkl) planes, 'a' is the lattice constant. The sharp peaks values are used to calculate the lattice parameter and grain size.

The calculated lattice constant for the dominant peaks of CdO nano crystalline thin films is averaged to a = 4.011A° which is close to the reported value a = 4.695A°.

The information on the strain ε as well as the crystalline size (D) was calculated from the full-width at half maximum (FWHM) β of the prominent XRD peaks using the Scherer-Bragg's relation Eq. (2).

$$D = k\lambda/\beta \cos\theta \qquad (2)$$

Where λ is the wavelength of the X- ray, β is the full width at half maximum of the corresponding peak of the XRD pattern.

Dislocation density and micro strain

Dislocation density δ and micro strain ε were calculated using the following equations (3), (4).

$$\delta = 1/D^2 \text{ lines / m}^2 \qquad (3)$$

$$\varepsilon = \lambda/D \sin\theta - \beta/\tan\theta \qquad (4)$$

The micro structural parameters like grain size, dislocation density, and strain for pure CdO nano crystalline thin films are listed in table 1. It shows that the average grain size reaches a maximum of 38.76 nm.

4. MORPHOLOGICAL ANALYSIS

The morphology and microstructure of CdO products were examined by SEM images. Figure shows the structures of the CdO nanoparticles which were grown in pure and doped thin films on the glass substrates. It can be seen that all the substrates were completely surrounded by CdO nanoparticles.

5. RESULTS & DISCUSSIONS

The existence of multiple diffraction peaks of (011), (111), (200), (220), (311) and (222) planes specifies the polycrystalline nature of the CdO compound with cubic structure (Ngamnit Wongcharoen *et al.* 2012). The obtained XRD patterns are shown in Fig.1.

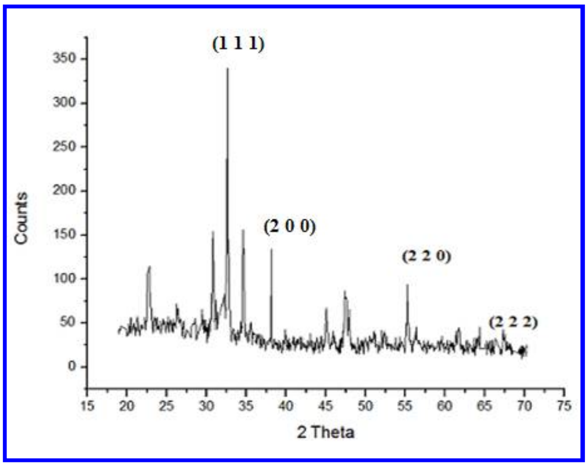

Fig. 1: XRD spectrum - CdO thin film

The XRD peaks indicate good crystalline nature of synthesized CdO. All CdO thin films have good adhesion to the substrate, are transparent, better uniformity, free from pinholes and are stable for a long

period when kept in the atmosphere (Gbadebo Taofeek Yusuf *et al.* 2016). The XRD pattern exposed diffraction peaks approximately at 33°, 55.5° and 68° of 2θ values, indicating the hkl values as (111), (220), and (222) which corresponds to polycrystalline having the characteristic peaks of face centered cubic structure of CdO (JCPDS Card No. 05-0640, 73-2245 and 78-065) (Gbadebo Taofeek Yusuf *et al.* 2016, Ngamnit Wongcharoen *et al.* 2012, Saha *et al.* 2007).

For Al doped CdO thin films with different concentrations of Al, it is capable of providing the cubic crystalline structure with polycrystalline nature in the same annealing temperature of 500 °C. The film did not stick to the substrate properly, formed as aggregates on the surface, thus producing numerous peaks in an amorphous fashion (Sivakumar *et al.* 2012). This performance can be related with the presence of Al-Cd compounds in the amorphous phase and with an increasing doped CdO grain size (Ngamnit Wongcharoen *et al.* 2012).

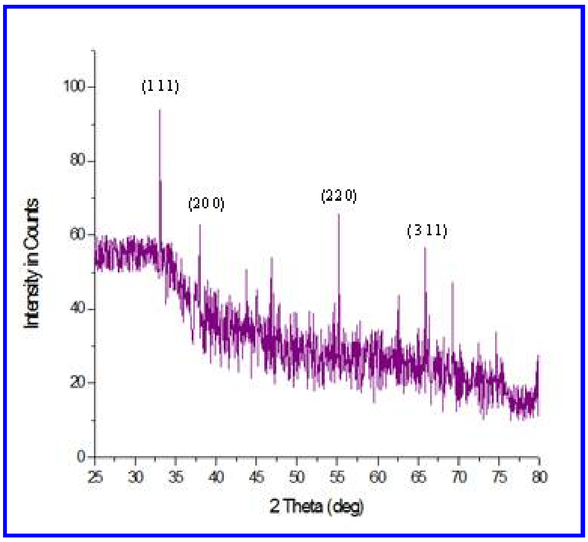

Fig. 2: (3wt %) Al: CdO thin film

Doping CdO films with Al causes small reduction in the intensity of all peaks and especially of the (220) plane. The doped CdO films have (111) plane as preferred orientation. Similar observation was also reported by Saha *et al.* 2007.

It is noticeable from the figure that the diffraction peak from (1 1 1) emerges as the strongest orientation for the doped film (Saha *et al.* 2007). The variation of the intensity of (1 1 1) peak in the Al doped CdO films is not uniform when Al concentration is increased uniformly from 3 to 5 wt% in the solution. This behavior implies that the variation of the crystallites size of the preferential orientation is not uniform due to the increase of aluminum concentration in the solution. The grain size of the crystallites is changed randomly for a regular raise of aluminum concentration .

The intensity of all peaks rapidly decreases and full width at half maximum (FWHM) decreases for the films with high Al content (5 %). The lattice parameter increases from 4.032 Å to 4.076 Å when Al percentage increases from 3 to 5 %.

Al was chosen in this work as dopant to organize and improve the properties of CdO thin films. Al^{3+} ion has three valence electrons and the ionic radius of Al^{3+} ion (0.68 Å) is slightly smaller than that of Cd^{2+} ions (0.95 Å), thus we expect that Al^{3+} ions doping in CdO will direct to enhancement in electrical conductivity by increasing electron concentration (Gupta *et al.* 2008). Since Al^{3+} has lesser ionic radius than that of Cd, there would be significant difference in its electrical property (Zheng *et al.* 2010). This is attainable by a shift in the optical band gap along with the enhancement in transparency of CdO films (Salunkhe *et al.* 2008). The effect of doping on the physical properties of CdO thin films was reported by Zheng *et al.*. The hkl and d value found for the Al doped CdO film deposited at different concentration (annealing temperature 500 °C) are agreeing well with the values found in JCPDS cards 05-0640 and 78-0653. The calculated value of lattice parameters, approximately for Al doped CdO film with 3wt% is a = 4.032A°. For the increased concentration of Al doping 5wt% a = 4.076A° is in very good agreement with the reported value of a = 4.695A°.

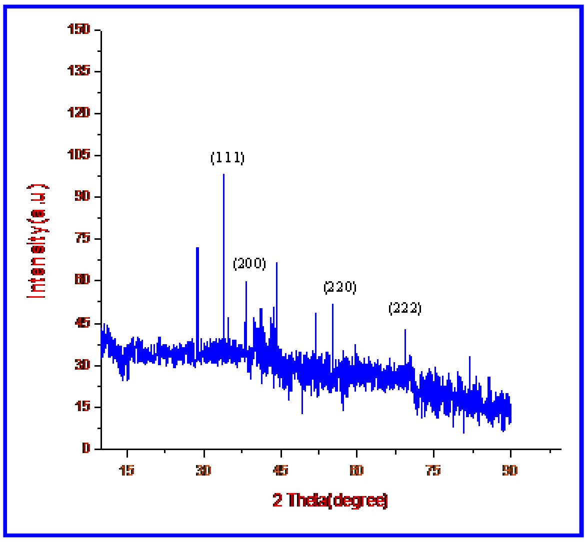

Fig. 3: (5wt %) Al: CdO thin film

Table 1. The Structural Parameters of pure CdO nano crystalline thin films

Film	$2\Theta_{deg}$	FWHM radians	Lattice strain	Dislocation density×10^{14} (δ)lines/m^2	Micro strain(ε)	Average crystalline size(D) nm
Pure CdO	15.2526 18.3321 22.6727 26.3868 29.0478	0.3444 0.1476 0.1968 0.5904 0.1476	0.0225 0.0080 0.0087 0.0223 0.0051	17.1319 03.1414 05.5651 49.9450 03.1105	9.0365 8.7565 6.8558 4.2314 5.5734	38.76
Al:CdO 3wt%	5.7385 18.2243 31.8419 66.5304 84.4461	0.5063 0.5196 0.1299 0.9092 0.2598	0.0882 0.0285 0.0041 0.0133 0.0029	37.18024 38.91642 2.401467 110.1068 8.524695	20.67552 6.48847 5.160884 1.422715 4.87948	28.148
Al:CdO 5wt%	27.4841 31.8421 45.5578 56.7029 66.3474	0.3897 0.1299 0.0974 0.2598 0.3897	0.0141 0.0041 0.0021 0.0045 0.0057	21.714637 2.401467 1.323002 9.205038 20.235832	1.50669 5.16084 3.738737 2.762732 2.219410	45.624

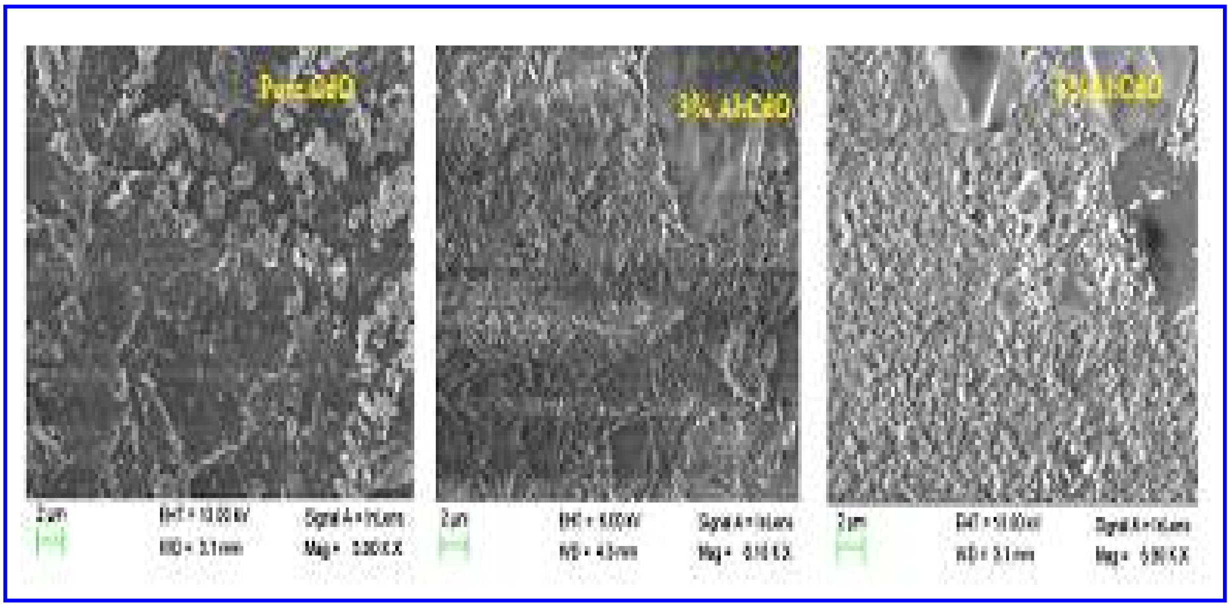

Fig. 4: SEM images for undoped and Al doped CdO thin films

A careful observation of pure CdO surface shows grains like mounts with no well defined boundaries it can be seen that grains clump together and hence do not display homogeneous distribution for undoped CdO samples and similar observations also reported (Aswani *et al.* 2014). One can clearly observe a rough surface with small grains. No pinholes or cracks are observed. The surface roughness is too high due to the changing grain size distribution on the surface of the film (Sukru Karatas *et al.* 2012). Average grain size could not be calculated due clumping (Colak *et al.* 2013).

The particle size is in few nanometer ranges and it exhibits needle like structure (Aswani *et al.* 2014) for Al doped CdO nanopowder.

It is observed that the doping of Al stimulate an obvious change in grain size. Furthermore, the influence of incorporation of Al on surface morphology of the samples can be clearly seen (Gupta *et al.* 2008). The thin films with 3% and 5 % doping concentrations are uniform and homogeneous, with few defects. The change of particle size can be attributed to the difference in the radius of ions between Cadmium and Al (Lokhande, 2004). The aluminum doped films show smooth surface compared to the Cadmium Oxide films.

6. BACTERICIDAL EFFECT ON GRAM-POSITIVE (BACILLUS CEREUS) BACTERIA

Antibacterial activity of CdO films were assayed by using the agar well diffusion technique. The Gram-positive bacteria, Bacillus cereus were grown on nutrient agar plates. Three different plates were used. Wells were made on the plates with the help of a well borer. The CdO nano thin films are dissolved in ethanol and prepared to the form of a solution and undoped, 3 wt% Al doped and 5 wt% Al doped CdO were added to the three wells. Likewise, the solutions of undoped precursor solution were taken on another plate.

In the first disc, which is impregnated with gram-positive bacteria (Bacillus cereus), undoped CdO and pure distilled water, as the control solution, were taken in 2 wells. The discs were incubated for 24 hours at 37 °C. After 24 hours the plates were visualized. Bindhu *et al.* reported that the undoped Cadmium Oxide nanoparticles inhibited the gram-positive bacteria Bacillus Cereus to a diameter of 13mm (Bindhu *et al.* 2016). For the undoped Cadmium Oxide, the diameter of Zone of inhibition is found to be 28mm (Fig. 2), which is comparatively an improved result to Bindhu *et al.*. From the figure, it is also observed that there is no zone of inhibition around the control solution, the distilled water.

The second disc displayed the inhibition zone of Al doped CdO solution. Fig. 3 reveals the level of inhibition effects against Bacillus cereus, which gives the Zone of Inhibitory action (ZOI) as 33mm for 3wt% Al doping and 35mm for 5wt% Al doping. It is obvious that the present study is the first work, in which we have used the Al dopant in 3wt% and 5wt%, and we have achieved the maximum value of antibacterial effect, identified so for.

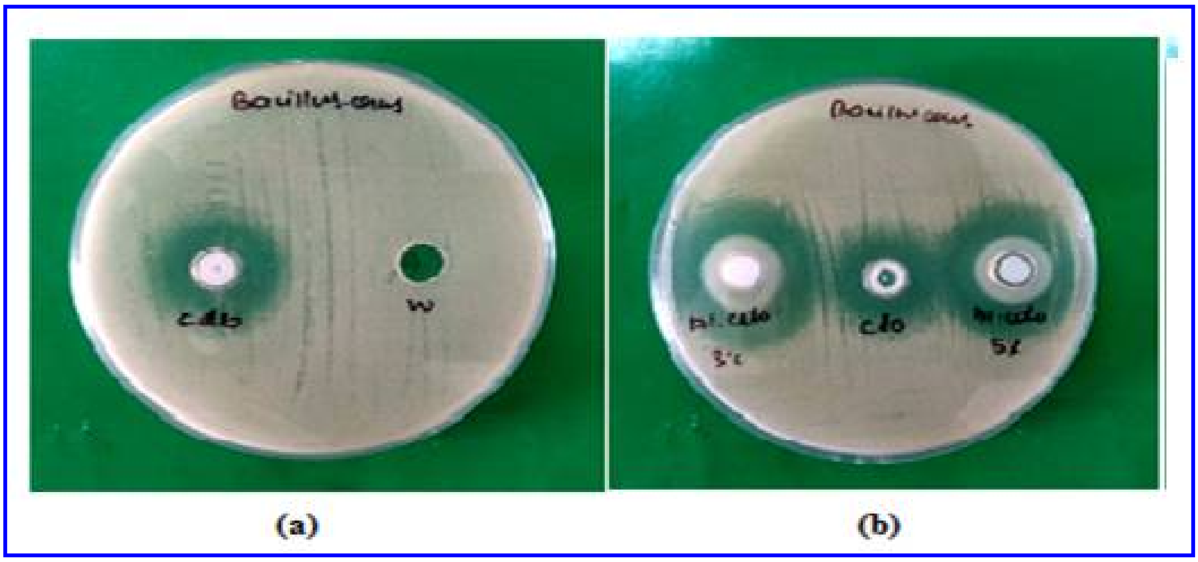

Fig. 5: Bactericidal effect of (a) undoped CdO (b) Al doped CdO on Bacillus cereus by well diffusion method

The present data demonstrate that a formation made with the organically stabilized nano CdO can be useful in the treatment of infectious disease caused by Bacillus cereus. It is implicit that microorganisms bear a positive charge (Zhang *et al.* 2009) which generates an "electromagnetic" attraction between the bacteria and the considered surface. Zhang H *et al.* realized that once the contact is made, the microorganism is oxidized and become lifeless immediately. Russell and Hugo concluded in their studies that a strong binding of nanoparticles to the outer membrane of E.coli causes the inhibition of active transport, dehydrogenase and periplasmic enzyme activity and eventually the inhibition of RNA, DNA and protein synthesis, leads to cell lysis (Russell *et al.* 1994). Generally, it is believed that nanomaterials release ions, which react with the thiol groups (-SH) of the proteins present on the bacterial cell surface. Such proteins extend beyond the bacterial cell membrane, permitting the transfer of nutrients through the cell wall. Nano materials diminish the proteins, lessening the membrane permeability and eventually causing the cellular death (Zhang *et al.* 2009). Wang *et al.*. accomplished in their work that in the aqueous system, both nanoparticles and bacteria had a tendency to combine, and the nanoparticles toxicities were mainly ascribed to the ions dissolved in the solutions (Wang *et al.* 2010).

7. BACTERICIDAL EFFECT ON GRAM-NEGATIVE (VIBRIO CHOLERAE) BACTERIA

The antibacterial activity of undoped and doped CdO solutions was analyzed to the gram-negative bacteria Vibrio Cholera also. This bacterium has not been taken much for analysis, for the antimicrobial assay. Our work is the first attempt to examine with the two different doping percentages of Aluminium.

The undoped CdO solution was assayed in the bacteria packed disc, which inhibits it to a zone diameter of 33 mm (fig. 2a). 3wt% Al doped CdO solution furnishes a Zone of Inhibition (ZOI) to the gram-negative bacteria Vibrio for about 42mm. The ZOI by 5wt% Al doped CdO solution appears to be 47mm (fig. 2b), which is the greatest value attained among the reviewed survey.

Ravichandran *et al.* demonstrated the maximum zone of inhibitory action (ZOI), emerged in the entire test when compared with undoped and La doped CdO. The antibacterial effect boosts with the increase in the La doping concentration may be of (i) the generation of Reactive Oxygen Species (ROS) (ii) Release of Cd+ ions and (iii) size of the nanoparticles (Ravichandran *et al.* 2016). In 2015, Ravichandran *et al.* explained the creation of ROS (Ravichandran *et al.* 2015) as follows:

$$\mathbf{CdO + h\upsilon \rightarrow h^+ + e^-}$$

$$\mathbf{h^+ + H_2O \rightarrow OH^- + H^+}$$

$$\mathbf{O_2 + e^- \rightarrow O_2^-}$$

$$\mathbf{O_2^- + H^+ \rightarrow HO_2^-}$$

$$\mathbf{H^+ + HO_2 + e^- \rightarrow H_2O_2}$$

$$\mathbf{H_2O_2 + O_2 \rightarrow OH^- + O_2 + OH^-}$$

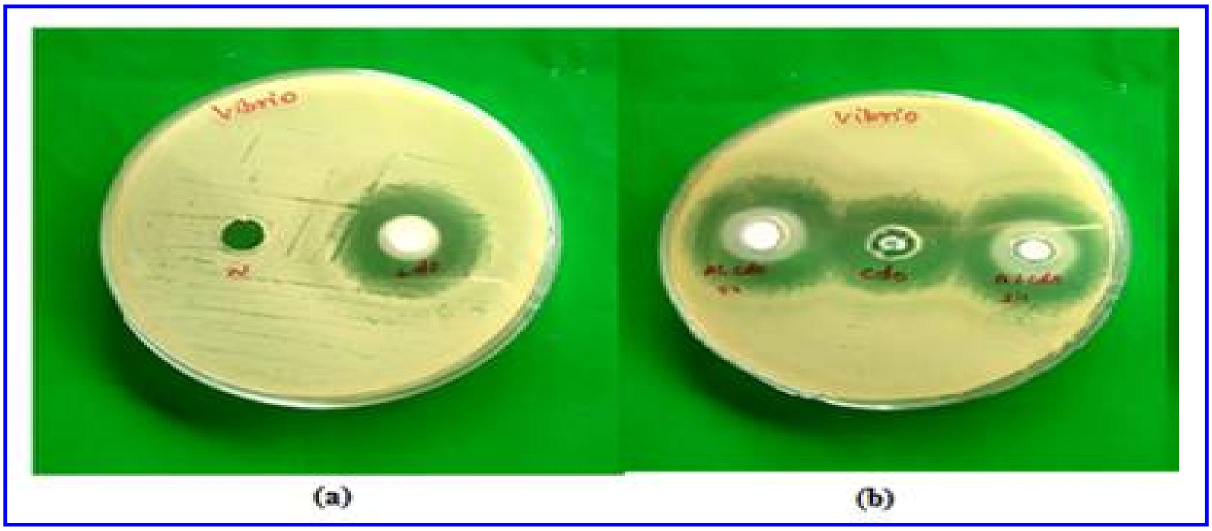

Fig. 6: Bactericidal effect of undoped and doped CdO on Vibrio by well diffusion method

The results revealed that AgNPs synthesized from C. bonplandianum demonstrated effective antibacterial activity in Gram-negative than in Gram-positive bacteria. It can be suggested that Gram-negative strains of bacteria E. coli and P. aeruginosa with thin cell wall is more susceptible to cell wall damage compared to Gram-positive strain bacteria S. aureus with a thick cell wall.

The toxic hydrogen peroxide released damages the structure of the bacteria cell membrane and depresses the activity of some enzymes which cause it to die eventually (Karthik *et al.* 2014). The basic mechanism of antibacterial action of the material states that the production of reactive oxygen species on the surface of these nanoparticles in light cause oxidative stress in bacterial cell and leads it to die. The ROS contains the most reactive hydroxyl radicals (OH), less toxic super oxide anion radical (O_2-). This damages the DNA, cell membrane etc., leading the cell to death. This is attributed to the electrostatic attraction between the negatively charged bacteria and the positively charged nanoparticles (Abdulrahman Syedahamed Haja Hameed *et al.* 2013).

Table 2. Zone of Inhibition

Bacterium	ZOI in mm by Undoped CdO	ZOI in mm by 3 Wt% Al Doped CdO	ZOI in mm by 5 Wt% Al Doped CdO
GRAM-POSITIVE (Bacillus cereus)	**28**	**33**	**35**
GRAM-NEGATIVE (Vibrio Cholera)	**33**	**42**	**47**

8. CONCLUSION

Nano crystalline thin films of undoped and Al doped Cadmium oxide were synthesized. The structural and morphological properties of CdO nano particles were investigated using an X-ray diffraction analysis and scanning electron microscopy respectively.

The present study reveals that the diameter of zone of inhibition is found to be more for gram-negative bacteria than gram-positive for undoped CdO and doped CdO.

The zone of inhibition values are stated in the Table 1. No much citation is found on these particular doping percentages of Al to CdO films, our work is the first one to demonstrate the result of maximum inhibition zones of both gram-positive and gram-negative bacteria.

It is found that there is an increase in the inhibition value for Al doped CdO when compared with other CdO films. As the doping percentage is increased the inhibition zone diameter is enlarged. The Cadmium Oxide nano thin films are capable of killing the gram-negative bacteria, Vibrio Cholerae, than the gram-positive bacteria Bacillus cereus comparatively.

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